Electronic Supplementary Material (ESI) for RSC Advances.

Linear PVA-DTPA-Gd Conjugate for Magnetic Resonance Imaging

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Experimental

Materials

Polyvinyl alcohol (M_w, 98% hydrolyzed polyvinyl acetate, molecular weight averaging 53000–63000 Da), Diethylenetriaminepentaacetic dianhydride (DTPAda) (>98%), Thiazolyl blue tetrazolium bromide (MTT, 98%), thionyl chloride, $GdCl_3 \cdot 6H_2O$, azo arsine, dimethyl sulfoxide (DMSO, 99.8%), dichloromethane, acetone and isopropanolamine were all purchased from Aldrich. D_2O was purchased from Fisher. Regenerated cellulose dialysis membrane (Spectra/Por 6, molecular weight cutoff 1000 Da) was purchased from Fisher. MilliQ water (resistivity>18.2 MΩ cm^{-1}) was generated using a Millipore MilliQ Academic Water Purification System.

Synthesis of PVA-DTPA

The operations were carried out under N_2 atmosphere *via* standard Schlenk techniques. PVA-DTPA was prepared as following: PVA (0.14g, 0.0032 mol) was dissolved in DMSO (2 ml). The solution was degassed with nitrogen gas in an ice bath for 30 min. Then DTPAda (0.25 g, 0.0006 mol) was added. The mixture was stirred for 24 h and was precipitated into acetone to give PVA-DTPA conjugate as a white powder (0.10 g, yield: 66%). The resulting polymer was purified by dialysis *via* 3000 M_w cut-off dialysis bag, and lyophilized to dryness, giving PVA-DTPA conjugate as white powder (0.24g, 62%).

Synthesis of PVA-DTPA-Gd

PVA-DTPA-Gd was prepared as following: PVA-DTPA (0.14 g) was dissolved in 20 mL H_2O. $GdCl_3 \cdot 6H_2O$ (155 mg, 0.42 mmol) dissolved in 1 mL H_2O was added, at the same time the pH value of the system was maintained around 5.5 using 1.0 M NaOH. Afterwards, the reaction solution was placed in an oil bath thermo stated at 70 ^oC for 12 h. The resulting reaction solution was cooled down to room temperature and was dialyzed against water for 48 h in a dialysis bag. The final product was obtained by lyophilizing the dialysis solution.

The stability study

The amount of Gd^{3+} released by PVA-DTPA-Gd conjugate was determined according to the previous literature [1]. In a typical procedure, 60 mg of PVA-DTPA-Gd was dissolved in 10 mL of phosphate buffer pH 7.4 (10 mM). The solution then was loaded into dialysis bags (MWCO 1000). The dialysis bag was immersed into corresponding buffer medium (150 mL) to study the Gd^{3+} release. At each time interval, 2.5 mL of exterior buffur medium was withdrawn and mixed with 2.5 mL of acetic acid-sodium acetate buffer solution and 2.5 mL of azo arsine solutions (0.01%), then, the solution was fixed to a 25 ml volumetric flask. The concentration of Gd(III) in the filtrate was determined for UV measurement at the wavelength of 656 nm. The concentration of Gd^{3+}

was determined based on a calibration curve. The accumulative release rate was calculated as follows:

$$\text{Accumulative release } \% = \frac{M_t}{M \times X} \times 100\%$$

Where M_t is the amount of Gd^{3+} at time point t, M is the total weight of the conjugate, X is the Gd^{3+} content in the corresponding conjugate (%).

[1] Feng Wang, Wenbo Wang, Yongfeng Zhu, Aiqin Wang. Evaluation of Ce (III) and Gd (III) adsorption from aqueous solution using CTS-g-(AA-co-SS)/ISC hybrid hydrogel adsorbent. Journal of Rare Earths, 2017, 35, 697-708.

MTT cytotoxicity assay

The MTT assay was introduced to study the cytotoxicity of the conjugate-based nanoparticles against HUVEC cells according to previous reports. HUVEC cells were seeded into a 96-well plate at 5×10^3 cells per well and were incubated in PRMI-1605 Medium (include 10% FBS) for 24 hours at 37 °C, 5% CO_2 in the air. After 24 h, the PVA-DTPA-Gd conjugate and free DTPA-Gd (equivalent Gd^{3+} dose, 5, 10, 20, 40, 60, 80 and 100 μg mL^{-1}) was added, respectively. The additional culture lasted for 24 h at 37°C. Before being incubated with MTT (5 mg/mL^{-1}, 20 μL per well), the medium was removed and cells were washed twice with ice-cold PBS. Then the solution was removed before adding dimethyl

sulfoxide (150 μL per well) into the wells to dissolve the formazane of MTT. The cell viability was calculated *via* ELISA plate reader (Thermo Fisher Scientific, MA, USA) with the ultraviolet absorbance at 490 nm. The optical density was used to calculate cell viability.

MRI Measurement

MRI experiments were conducted by a 0.5 T nuclear magnetic resonance analyzer (MesoMR23-060H-I, Suzhou Niumag Analytical Instrument Co., China). The MRI samples were prepared by drawing PVA-DTPA-Gd solutions at different gadolinium concentration into 1 mL disposable poly (propylene) syringes. For the *T1* measurement, inversion-recovery fast spin-echo sequence with 8 inversion times (*TI*; ranging from 10 ms to 4 s) was employed. Meanwhile, for the *T2* measurement, multislice multi-echo sequence was employed. [Others parameters: *TR* = 6 s (for *T1*) and 2 s (for *T2*); matrix size = 128 x 128; field of view = 40 x 40 mm; slice thickness = 4 mm). The r_1 and r_2 values were calculated from the slop of curve-fitting result of $1/T_1$ and $1/T_2$ (s^{-1}) versus the Gd concentration (mM).

Hemolysis assay

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Gansu Agricultural University and approved by the Animal Ethics Committee of Gansu

Agricultural University. The fresh blood of 5.0 mL was drawn from rabbit and immediately placed into saline containing centrifuge tube. The tube was centrifuged at 2000 rpm for 10 min and the supernatant was discarded. The obtained precipitate was repeatedly washed and centrifuged until the supernatant was substantially colorless. The precipitated red blood cells were mixed with physiological saline to prepare a 2% (v/v) cell suspension. A series of solutions (0.2 mL) of different mass concentrations were placed in the centrifuge tube to which an equal volume of 2% (v/v) red blood cell suspension was added. Another 2% (v/v) red blood cell suspension (0.2 mL) was added to an equal volume of distilled water and physiological saline as a positive control and a negative control, respectively. The all tubes were thoroughly mixed and incubated for 1.0 h in a 37 °C incubator. Then, the all tubes were centrifuged at 3000 rpm for 10 min and the supernatant (100 uL) was pipetted into a 96-well plate. The OD value of each well at a wavelength of 540 nm on a 96-well plate was measured using a microplate reader. The hemolysis rate is calculated using the following formula: Hemolysis rate % = [(sample OD -negative OD) / (positive OD - negative OD)] × 100%. Each concentration was set to three parallel groups.

Characterization

The morphologie of the obtained sample is examined by using transmission electron microscopy (TEM, FEITECNAIG2TF20, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend-600 instrument. The chemical shifts are reported in ppm relative to the solvent residual peak. A Bruker IFS 66v/s IR spectrometer (Bruker, Karlsruhe, Germany) was used for the Fourier transformed infrared (FTIR) analysis in the range of 400–4000 cm^{-1} with the resolution of 4 cm^{-1}. The molecular weights and distributions were obtained by Gel Permeation Chromatography (GPC, Waters Alliance GPCV 2000 chromatograph). The composition and structure were characterized by TGA (NETZSCH, STA449F3) and X-ray photoemission spectroscopy (XPS, Escalab 250Xi). The UV-vis spectra was determined by ultraviolet spectrophotometry (Tu-1901 UV/vis spectrophotometer). Dynamic light scattering (DLS) were measured at 25 °C using a Malvern Zetasizer NanoZS Instrument equipped with a 4 mW He-Ne solid-state laser operating at 633 nm. The samples were dissolved in phosphate buffered saline (PBS 10mM). Backscattered light was detected at 173° and the mean particle diameter was calculated from the quadratic fitting of the correlation function over thirty runs each of ten seconds duration.

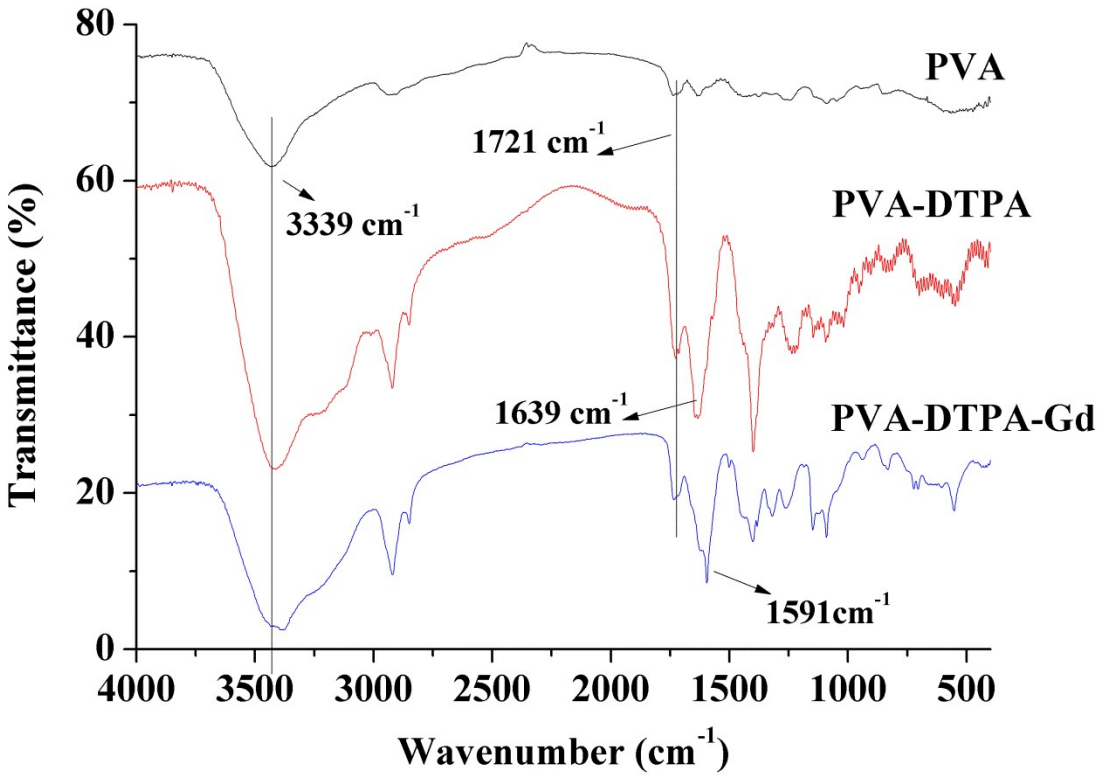

Fig. S1 FTIR spectra of PVA, PVA-DTPA and PVA-DTPA-Gd.

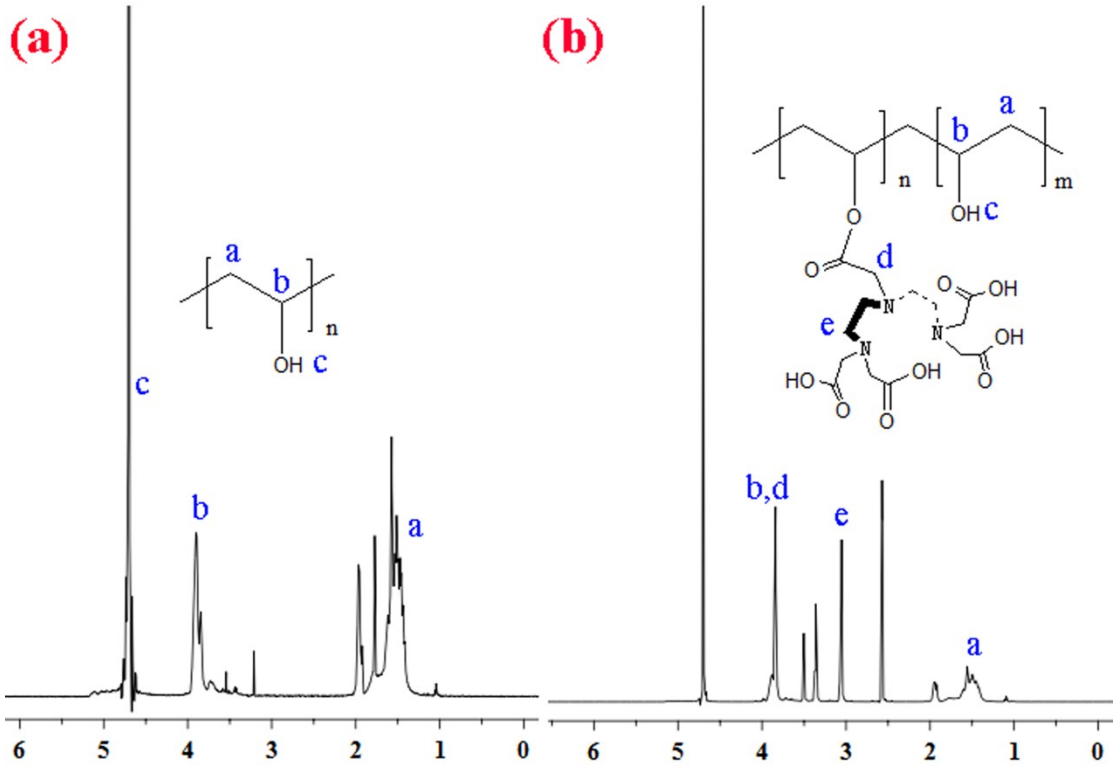

Fig. S2 NMR spectra of PVA(a) and PVA-DTPA(b).

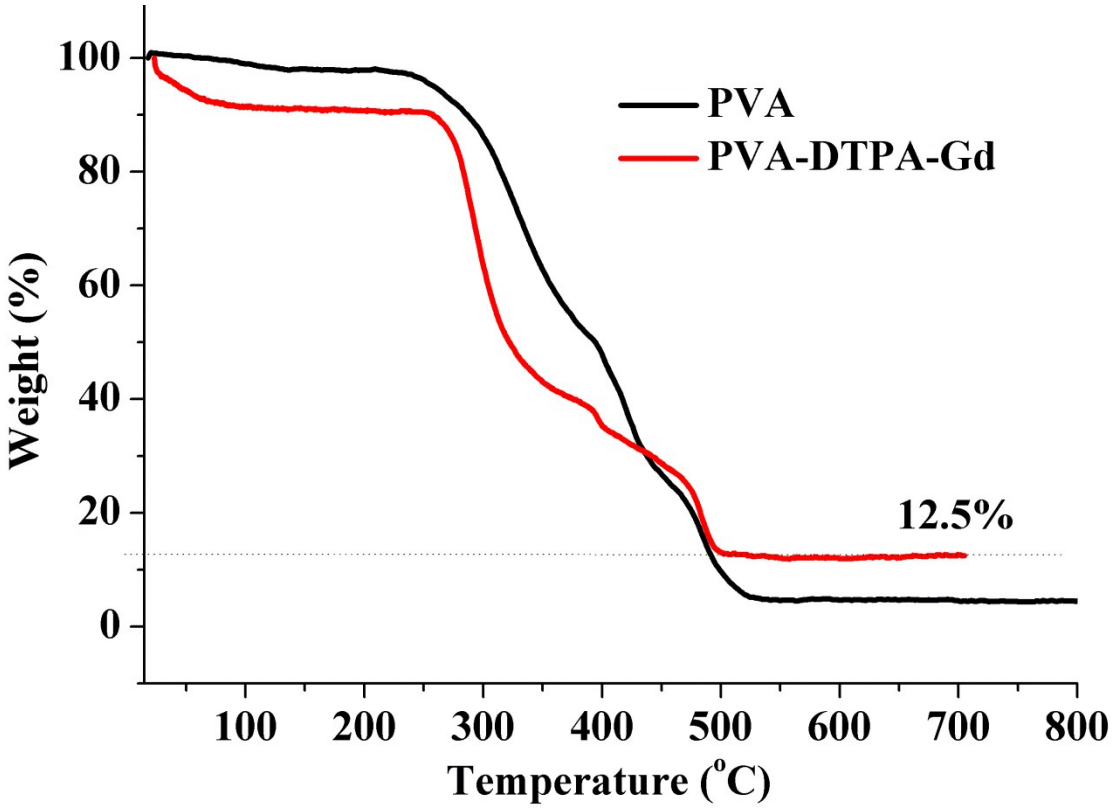

Fig. S3 TGA curves of PVA and PVA-DTPA-Gd.

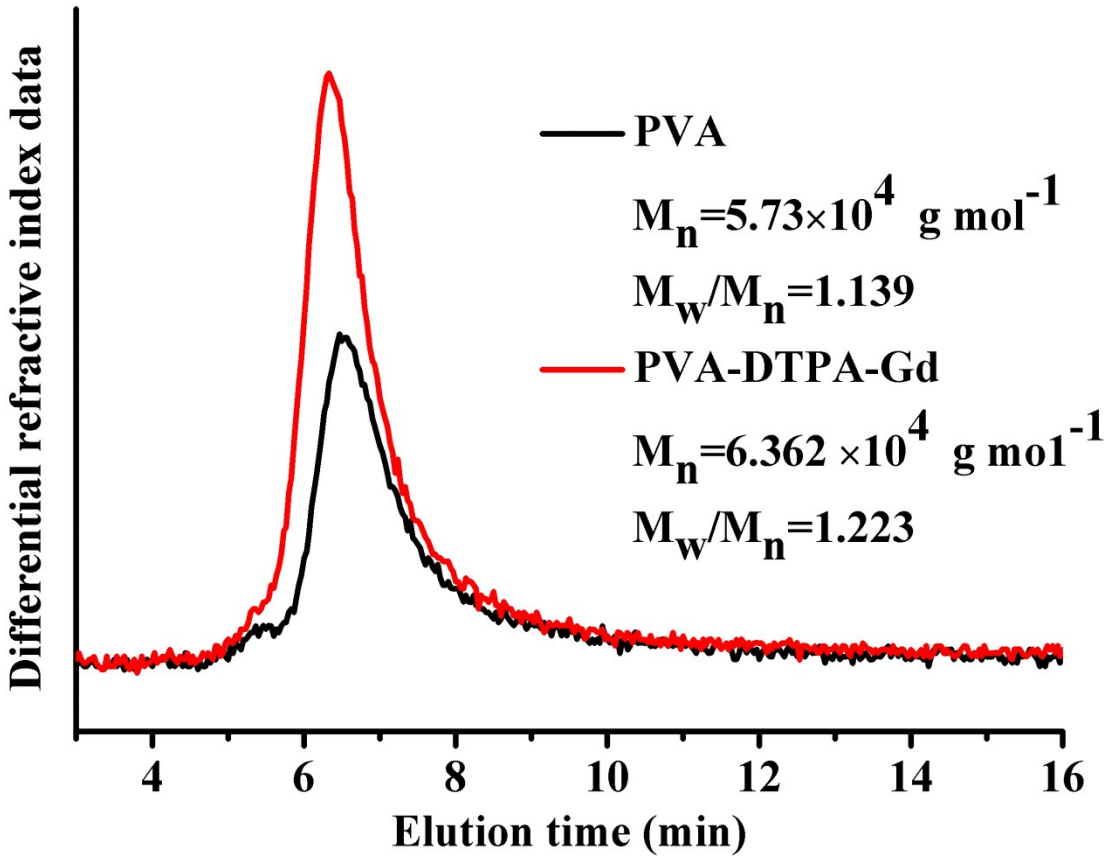

Fig. S4 GPC trace of PVA and PVA-DTPA-Gd conjugate.

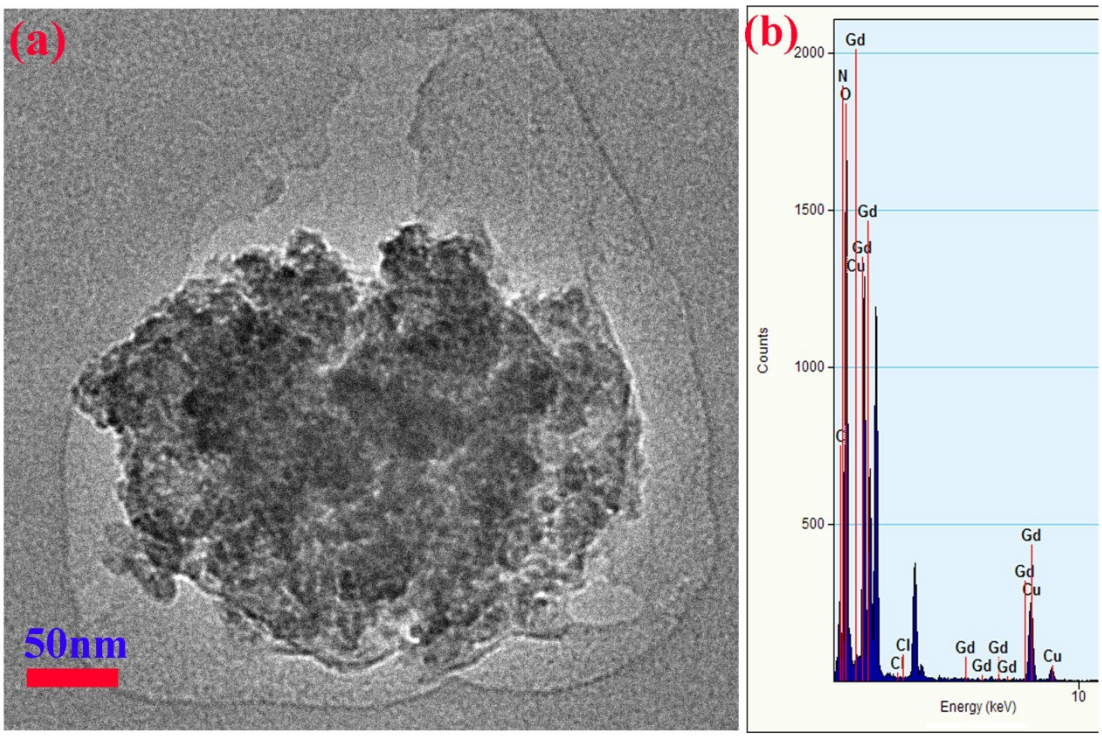

Fig. S5 TEM image (a) and EDX curve (b) of PVA-DTPA-Gd conjugate.

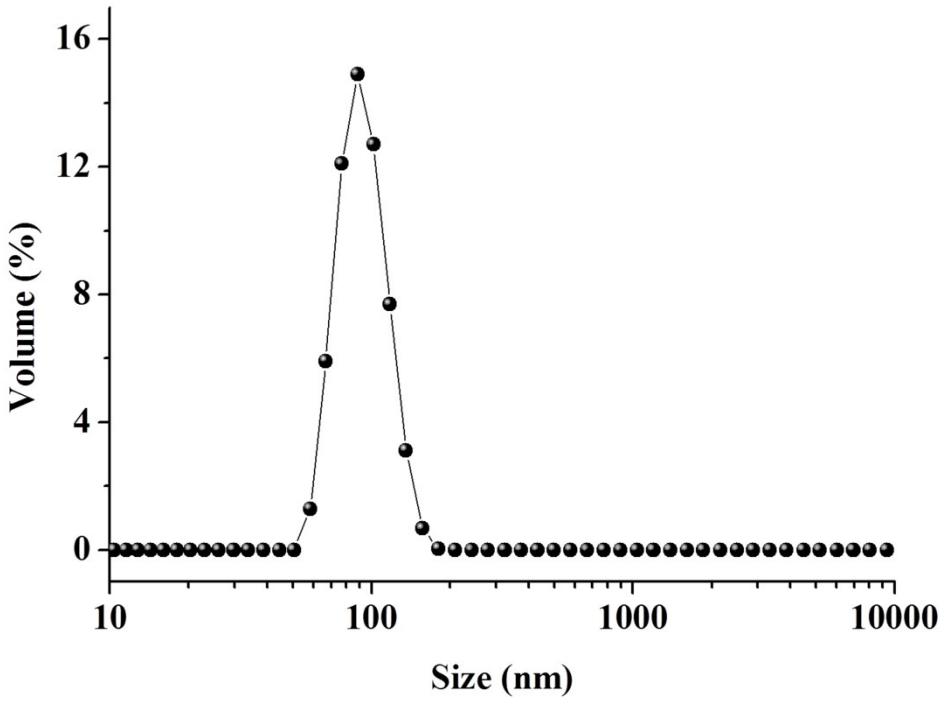

Fig. S6 DLS curve of PVA-DTPA-Gd conjugate.

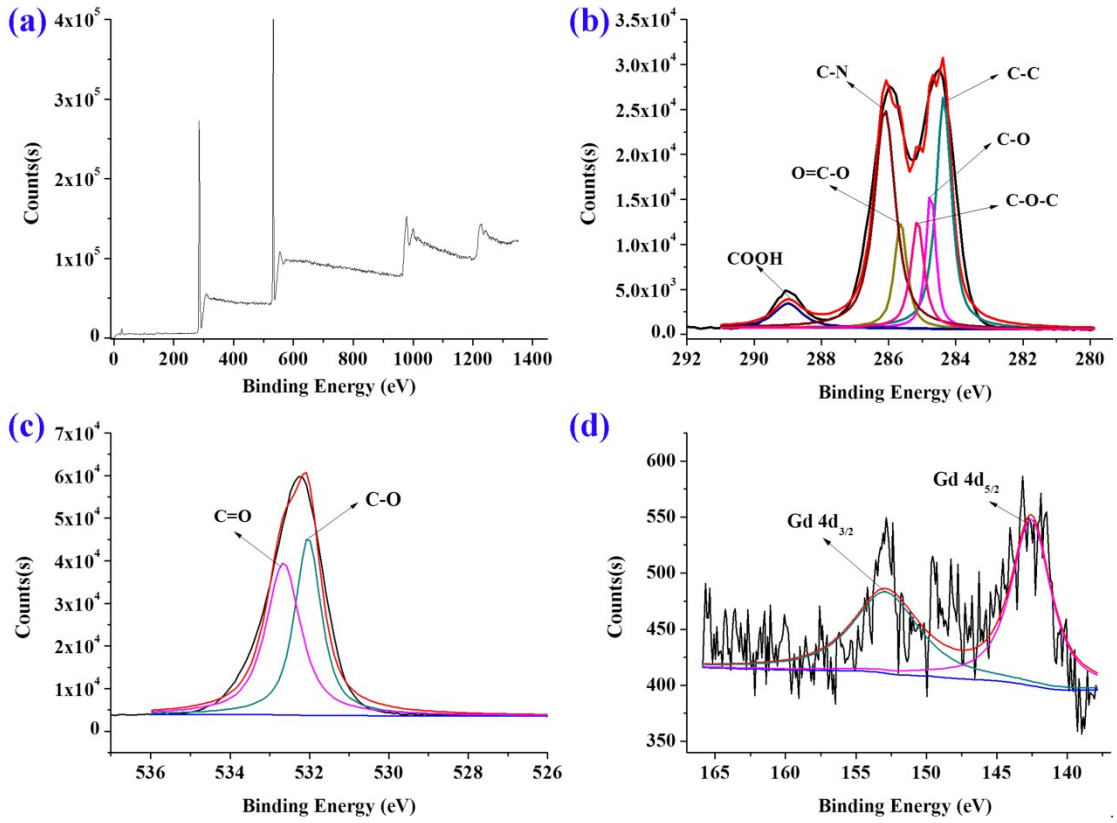

Fig. S7 (a) XPS survey, (b) C1s, (c) O1s, (d) Gd4d XPS spectra of PVA-DTPA-Gd conjugate.

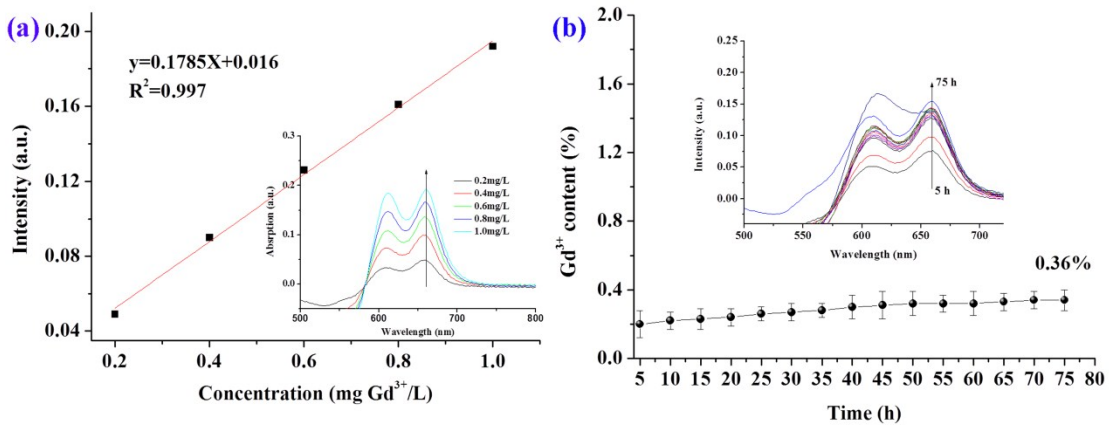

Fig. S8 The calibration curve (a) and the release of Gd^{3+} from PVA-DPTA-Gd conjugate at different time interval (b).

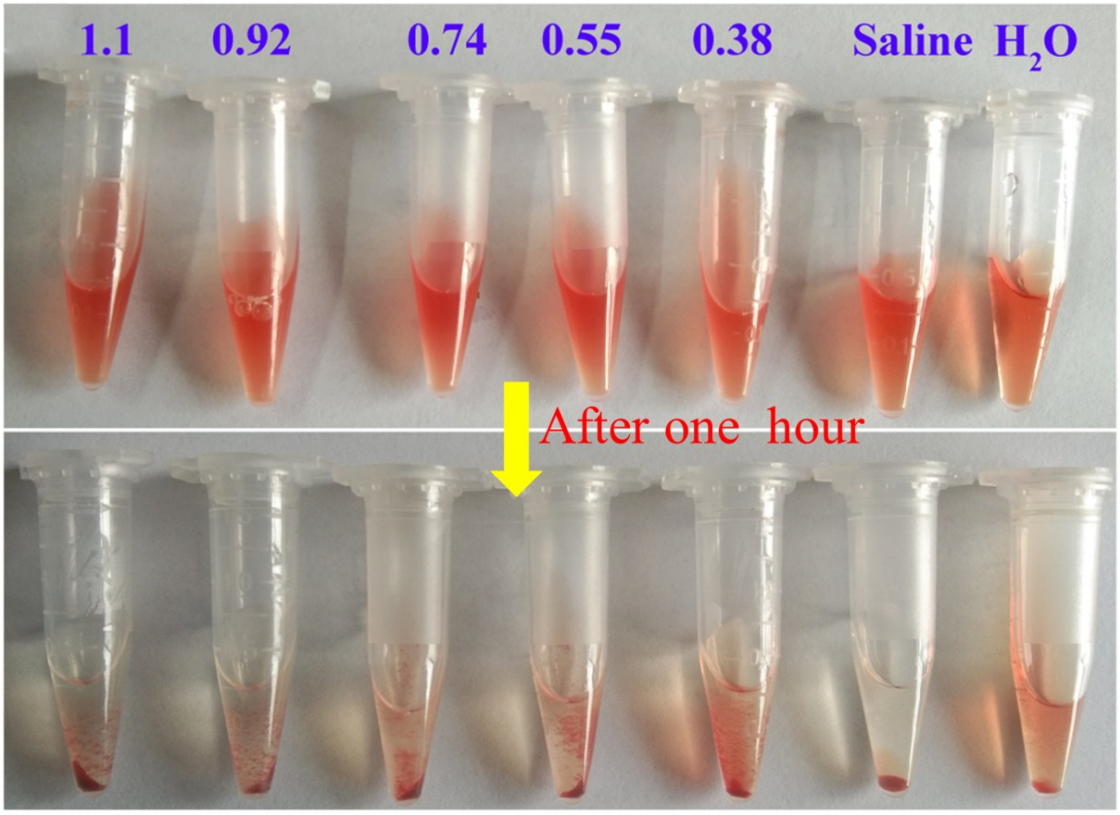

Fig. S9 Erythrocyte hemolysis resulted of PVA-DTPA-Gd conjugate.

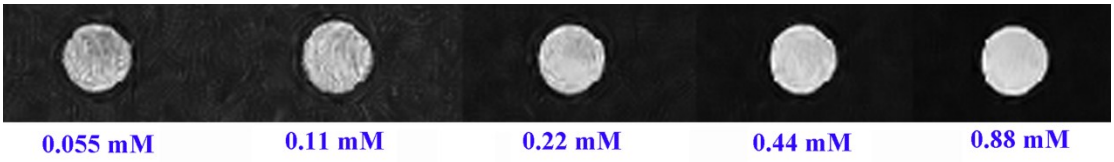

Fig. S10 The MRI images of free DTPA-Gd at various concentration.